(12) UK Patent Application (19) GB (11) 2 293 241 (13) A

(43) Date of A Publication 20.03.1996

(21) Application No 9418463.7

(22) Date of Filing 14.09.1994

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(51) INT CL[6]
G01N 27/06 , C12Q 1/04 , G01N 33/04

(52) UK CL (Edition O)
G1N NBMA NBPE N25B N25C3T N25DX N25E1
U1S S1294

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(58) Field of Search
UK CL (Edition N) G1N NBMA NBMK NBMM NBPC NBPE NBPX
INT CL[5] C12Q 1/04 , G01N 27/06 33/04 33/487
Online : WPI

(54) Detection of microbiological contamination of liquid or semi-liquid substances

(57) A method and device for testing a liquid or semi-liquid substance for microbiological spoilage. In the method, spaced first and second electrodes are contacted with the substance in undiluted form, an electrical current is supplied between the electrodes, the voltage or current between the electrodes is monitored, and a signal is produced when the said voltage or current exceeds or falls below, respectively, a predetermined value indicative of contamination. A test device for carrying out this method comprises an insulating substrate, and first and second carbon electrodes formed on the substrate by screen process printing using a carbon ink, at least end portions of the electrodes at one end of the test device being accessible electrically for connection to an electrical test apparatus by a severable electrical connection.

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Fig.1(a)

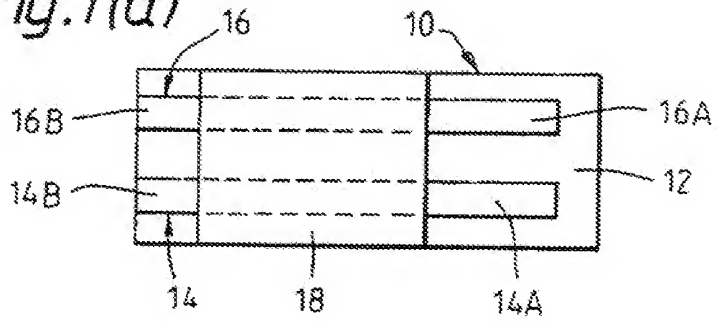


Fig.1(b)

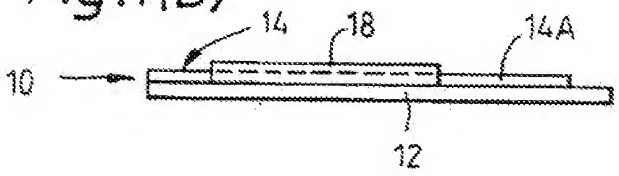


Fig. 2

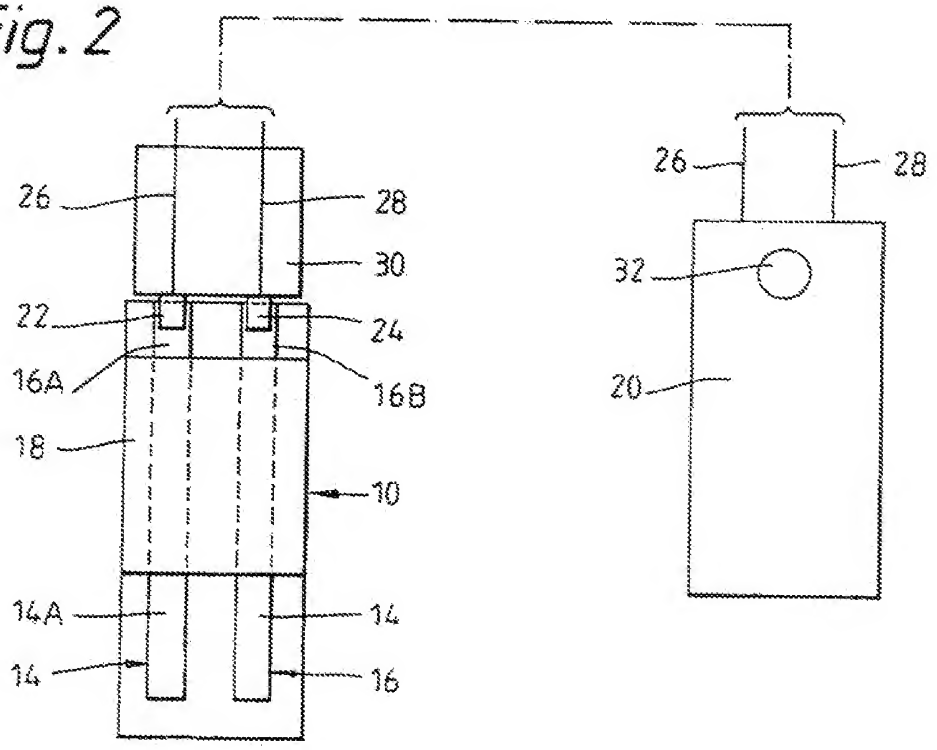
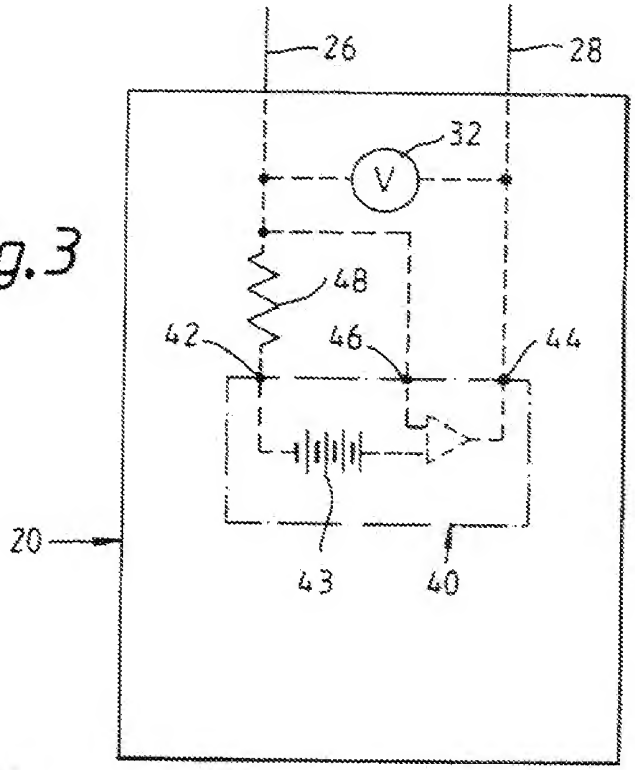


Fig.3



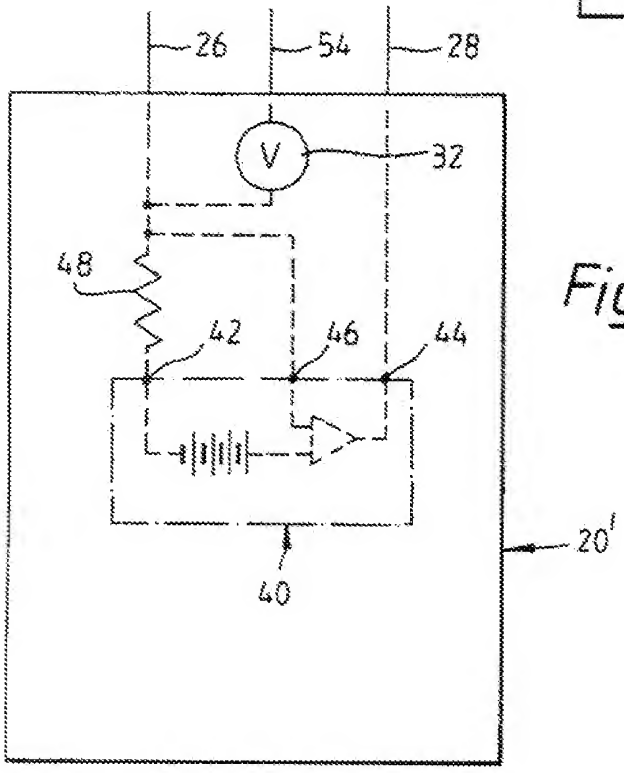
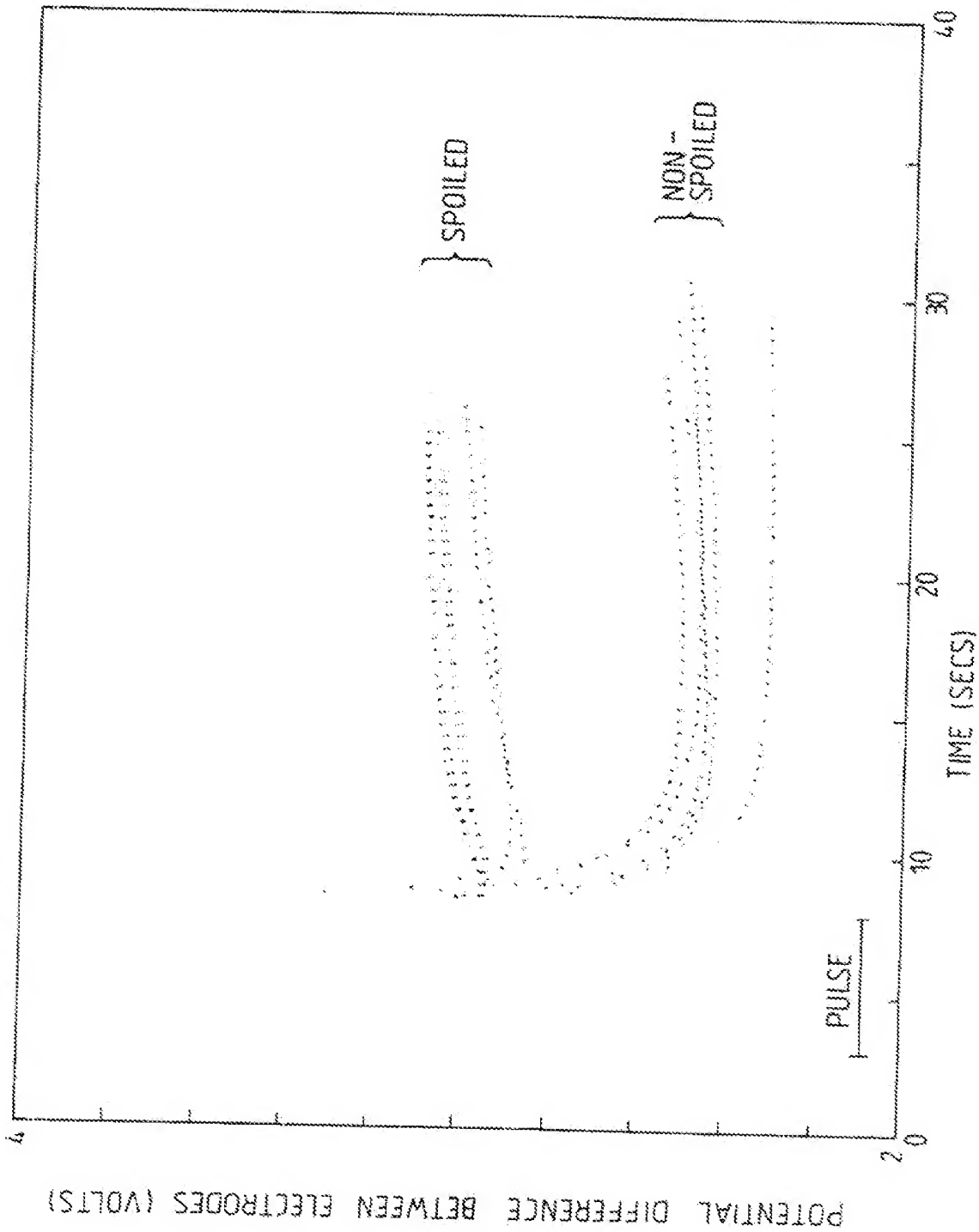


Fig.8

Fig. 4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NON-SPOILED
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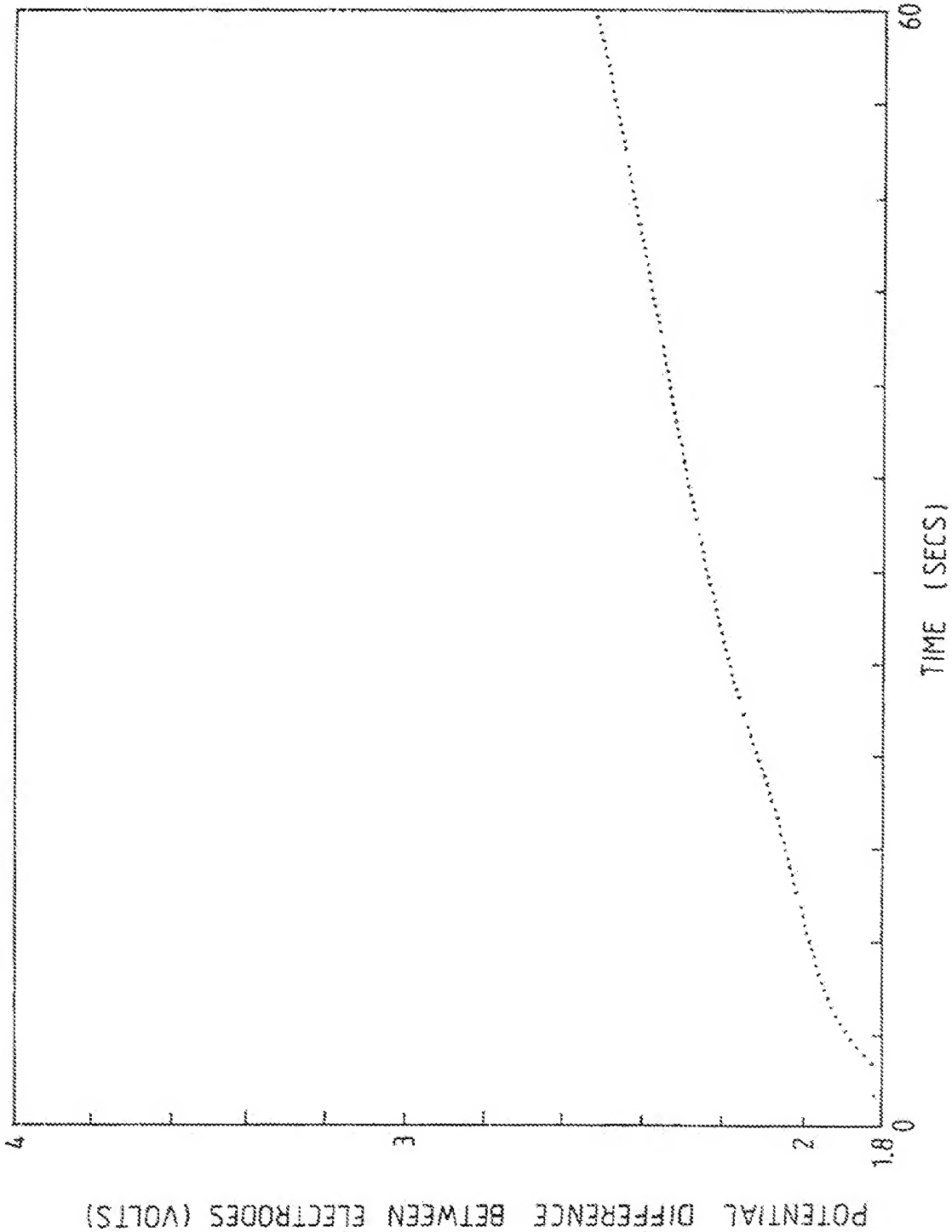
30
40
2
4
POTENTIAL DIFFERENCE BETWEEN ELECTRODES (VOLTS)

Fig.5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1.8
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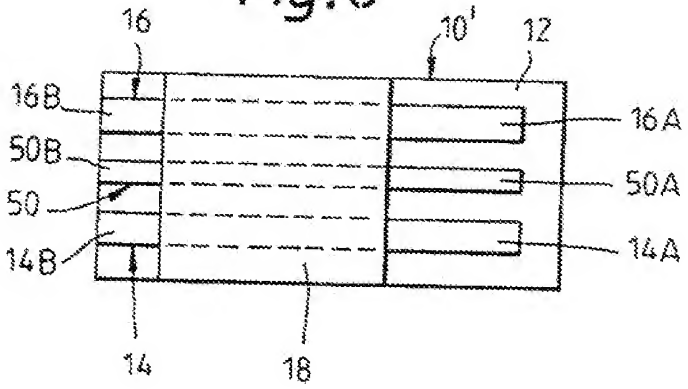
60
TIME (SECS)
POTENTIAL DIFFERENCE BETWEEN ELECTRODES (VOLTS)

Fig. 6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10'
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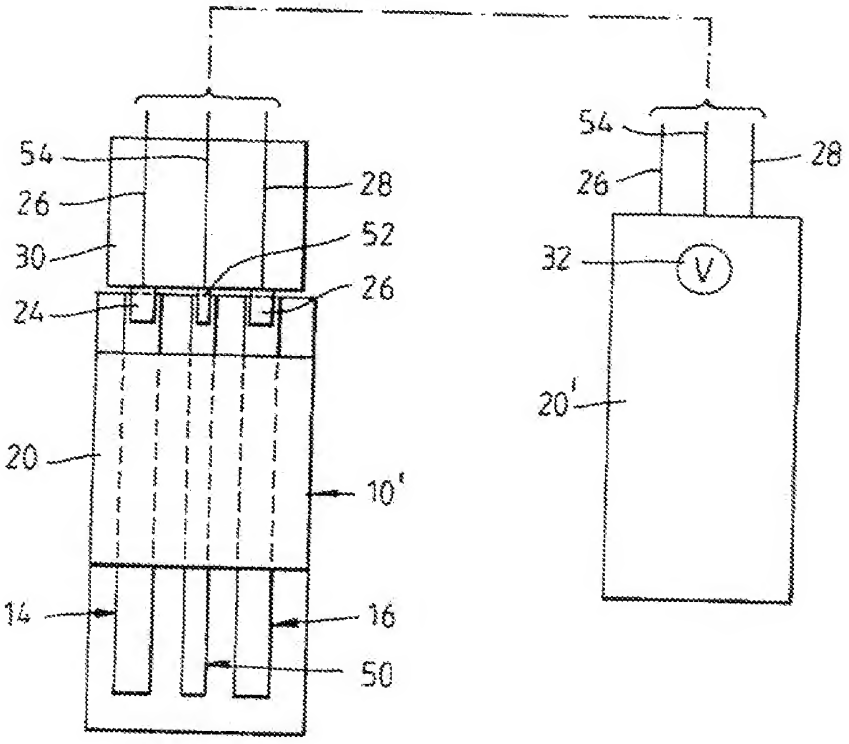
16B
16A
50B
50A
50
14A
14B
14
18

Fig. 7


54
28
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26
24
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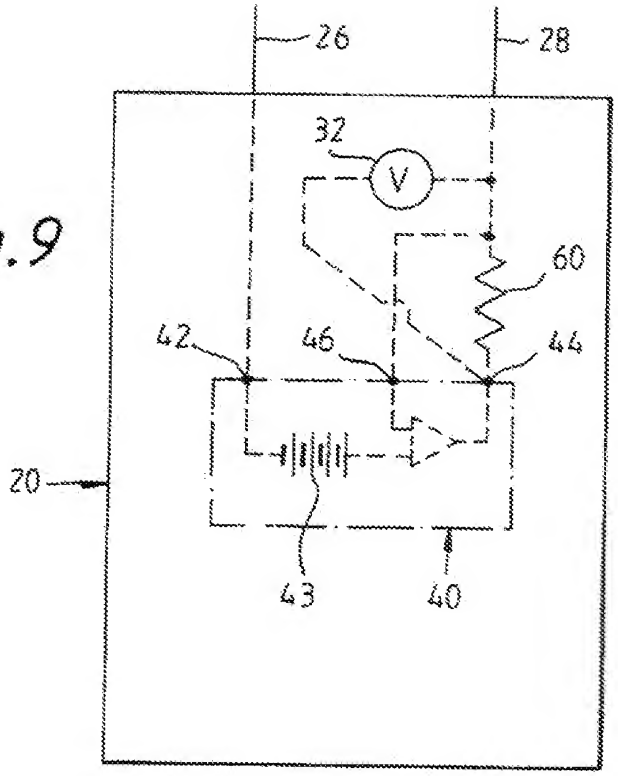
10'
14
16
50
54
28
26
32
V
20'

Fig.9



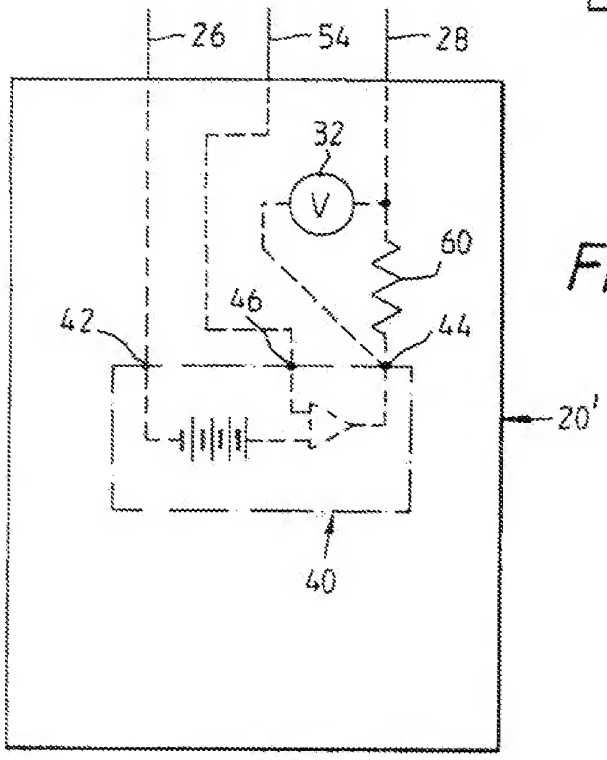


Fig.10

DETECTION OF MICROBIOLOGICAL CONTAMINATION OF LIQUID OR SEMI-LIQUID SUBSTANCES

The present invention relates to the detection of microbiological contamination of liquid or semi-liquid substances especially, but not necessarily, food products having a substantial liquid content.

The invention is concerned particularly with detecting contamination of such food products which occurs as a result of growth of aerobic micro-organisms. This is a particular problem nowadays where food products are often treated, thermally or otherwise, to increase the period of time for which the packaged food product can be stored before any significant deterioration occurs, that is to say, its shelf life. It is necessary to know that after the treatment and subsequent packaging the product is free of micro-organisms which could subsequently multiply and cause spoilage. A good example of the type of food product for which the present invention is appropriate is ultra-heat-treated (UHT) milk. Following UHT treatment, milk is aseptically packaged into foil lined cardboard cartons. Sample packs from each production batch are removed for quality control testing, and the remaining packs of the batch are placed in quarantine awaiting the results of the tests. The percentage of contaminated packs (if the packaging operation is to blame) is usually low, and may be less than 1% of production. Moreover, the level of microbial contamination in each contaminated pack may be only 1 or 2 organisms. Thus, in order to ensure detection of contamination within a production batch, a number of sample packs are removed from the batch and incubated at elevated temperatures (e.g 30°C) for several days, after which they are analysed for the presence of micro-organisms.

Because of the importance of ensuring that contaminated product does not reach the consumer, considerable attention has been given to ways of detecting the presence of micro-organisms in the sample packs after incubation, in a manner which is rapid, cheap and reliable. To date, no satisfactory method has been developed which satisfies all these requirements to at least a substantial degree. The methods currently in use are generally reliable, but are not necessarily rapid or cheap and certainly not both. Another problem with many existing techniques is that they require removal and manipulation of some of the contents of each sample pack, so allowing contamination to occur which may later be detected. By falsely indicating contamination this may require a significant quantity of the product to be disposed of unnecessarily.

According to one aspect of the present invention there is provided a method of detecting microbiological contamination of a liquid or semi-liquid substance, the method comprising contacting spaced first and second electrodes with the substance in undiluted form, supplying an electrical current between the electrodes, monitoring the voltage or current between the electrodes, and producing a signal when the said voltage or current exceeds or falls below, respectively, a predetermined value indicative of contamination.

Applicants have discovered that it is advantageous to vibrate the test substance or the electrodes while testing is proceeding. A preferred frequency of vibration is about 5 vibrations per second, but a wide range of frequencies may be used.

For aerobic organisms the step of detection may be based on an electrochemical measurement of the residual oxygen level within the substance being tested. Growth of

the organisms results in the consumption of oxygen which is exhibited as an increase in potential between the first and second electrodes. Applicants have found that the potential difference which is sustainable between the electrodes is markedly greater if the substance is contaminated than if it is uncontaminated. UHT milk is a good example of a product liable to aerobic contamination, although Applicants believe that the method can be applied to the detection of contamination of many other low acid food products.

Particularly when applied to the detection of contamination in UHT milk, the method of the invention may include the step of incubating the product prior to testing. For some applications of the invention however, Applicants believe that this additional step may not be necessary.

The invention also provides a system for carrying out the above method, and a test device adapted for use in the system in conjunction with a suitable electrical test apparatus. A preferred such test device is disposable, i.e it is proposed for single use only, and comprises an insulating substrate, and first and second spaced carbon electrodes formed on the substrate by screen process printing using a carbon ink, at least end portions of the electrodes at one end of the test device being accessible electrically for connection to a said test apparatus by a severable electrical connection. The severable electrical connection preferably has the form of a socket carrying first and second resilient electrical terminals adapted for making connection with the end portions of the electrodes when the said one end of the test device is inserted into the socket.

The substrate is preferably in the form of a sheet of an insulating material. Suitable materials include

cartonboard, and polymers such as polyvinyl chloride, high impact polystyrene, polyethylene tetraphthalate, polycarbonate and polypropylene. Carbon ink is a commercially available material having a high conductivity.

For a better understanding of the present invention, and to show how the same may be carried into effect, reference will now be made by way of example to the accompanying drawings in which:

Figs.1a and 1b show respectively in plan view and side elevation a disposable test device for use in a microbiological detection system in accordance with the present invention.

Fig.2 shows the detection system to comprise the test device of Fig.1 when in association with an electrical supply and test apparatus;

Fig.3 shows circuit detail of the electrical supply and test apparatus;

Fig.4 is a graph of the potential difference across the electrodes of the test device when plotted against time for seven test samples;

Fig.5 similarly shows the graph of electrode potential difference against time for a further test sample;

Fig.6 is a view corresponding to Fig.1a of a modified test device;

Fig.7 is a view corresponding to Fig.2 of a detection system employing the test device of Fig.6;

Fig.8 is a view corresponding to Fig.3 showing circuit detail of the supply and test apparatus of Fig.7 when arranged for galvanostatic operation;

Fig.9 similarly shows a supply and test apparatus when arranged for potentiostatic operation in conjunction with a test device having two working electrodes only; and

Fig.10 similarly shows a supply and test apparatus which is arranged for potentiostatic operation in conjunction with a device having two working electrodes and a reference electrode.

Referring now to Figs. 1a and 1b, a disposable test device 10 has a substrate formed of a rigid rectangular sheet 12 of an electrically insulating material such as cartonboard or a suitable polymeric material. Two electrically conductive spaced carbon tracks 14,16 are laid down on the substrate by screen process printing using a carbon-containing ink, such as that available from Coates-Lorilleux under their reference 26-8203, and these tracks respectively form cathode and anode electrodes for the device as is described below.

The test device is intended to be dipped into the surface of a liquid or semi-liquid substance under test (e.g. a carton of UHT milk from a production batch), and for consistency of the test results a rectangular mask 18 of a moisture impermeable material is formed as a coating on an intermediate part of the substrate, so as at one end of the device to leave exposed portions 14A, 16A of the electrodes which are accessible to the product. The portions 14A, 16A accordingly provide predetermined areas of electrical contact with the test substance when the device is used. In the example shown the portions 14A, 16A are 20mm long by 5mm wide.

In addition to the portions 14A, 16A, further portions 14B, 16B of the electrodes 14, 16 are left exposed by the mask 18 at the opposite end of the device. As is shown diagrammatically in Fig.2, these additional exposed portions provide for connection to an associated electrical supply and test apparatus 20, by means of spring clips 22, 24 and flexible leads 26, 28 carried from a piece 30 of rigid insulating sheet which may be held by

the user between finger and thumb. The supply and test apparatus has a voltmeter 32 for indication purposes.

Fig.3 shows circuit detail of the apparatus 20. The apparatus has an operational amplifier 40 having terminals 42, 44 and 46 and connected as shown in relation to a supply battery 43. The terminal 42 is connected to lead 26 and thence to cathode 14 via a resistor 48, terminal 46 being connected directly to that lead. Terminal 44 is connected directly to lead 28 and thence to anode 16, and the voltmeter 32 is connected between the leads 26, 28 to provide a measurement of the voltage across the electrodes.

A first series of experimental tests involved seven sample cartons of UHT milk taken from the same production batch. Three of the samples were uncontaminated, but the remainder were inoculated with E.Cloacae and incubated for 16 hours at 30°C to grow the contaminant.

For each test the test device 10 was dipped into the milk sample until the exposed portion 14A or 16A of each electrode was wholly immersed and therefore presented a surface area of 100mm^2 to the milk.

The electrodes were then conditioned by application of a d.c. pulse of 800 uA of 5 seconds duration from the supply and test apparatus 20, it being noted in passing that the current density of this conditioning pulse at each electrode was 8 uA mm^{-2}.

Following the conditioning pulse the test current was reduced to 16 uA and maintained at that level for a further 20 seconds. The density of this current at each electrode was 0.16 uA mm^{-2}. Fig.4 shows the variation of the voltage across the electrodes 14, 16 as sensed by the voltmeter 32. From that figure it will be seen that by the end of the conditioning period the voltage for each test had attained a substantially constant steady state

level, and this level was maintained for the remainder of the test. It will further be seen that the steady state levels of all the contaminated samples were substantially higher than those of all the uncontaminated samples, and Applicants believe that this difference in levels can be used to provide an indication of microbiological spoilage, or lack of it, of a liquid or semi-liquid product such as UHT milk.

Fig.5 illustrates the effect of the conditioning pulse by showing the voltage variation which occurred during a test made on a typical uncontaminated sample of UHT milk using the same test procedure as above but without the conditioning pulse. It will be seen that the voltage across the electrodes 14, 16 rises steadily during the whole 60 second duration of the test, making it unsuitable as a rapid indication of microbiological spoilage.

In addition to conditioning using a high current pulse as described in a previous paragraph, Applicants have discovered that the detection of microbiological spoilage can be ensured if the electrodes (preferably) or the test sample are vibrated during testing. The frequency and amplitude of the vibration may be varied within wide limits, but Applicants have found that a frequency of about 5 cycles per second and an amplitude of about 10mm is suitable. The effect of the vibration is to reduce the average voltage level across the electrodes. Tests with both contaminated samples and uncontaminated samples are effected in this way, but it is found that the average voltage reductions for the contaminated samples are less than those for the uncontaminated samples, so further differentiating the two conditions to a degree which allows reliable identification irrespective of the temperature of the

samples. In the latter respect it is to be noted that the average voltage levels are to some extent temperature-dependant, there being a reduction of about 7% in the voltage levels given by samples tested at 10°C and 40°C respectively.

The detection system of Fig.2 and its component parts (Figs. 1 and 3) employ two (only) electrodes and a constant impressed current for testing. However, three electrodes, i.e. two working electrodes and one reference electrode, and constant voltage conditions rather than constant current conditions may be employed. Fig.6 is a view corresponding to Fig.1(a) of a three electrode test device 10', and Fig.7 shows the detection system employing that device together with a modified supply and test apparatus 20'. The additional electrode, denoted 50, has exposed parts 50A, 50B corresponding to the parts 14A, 16A and 14B, 16B of the working electrodes 14, 16. The associated spring clip and lead are denoted 52 and 54 respectively.

Fig.8 shows circuit detail of the electrical supply and test apparatus 20' when arranged to drive a constant current through the working electrodes 14, 16. The circuit arrangement is the same as that of Fig.3 (and the same references are used to denote corresponding parts), but the voltmeter 32 is connected between the leads 54 and 26 so as to measure the voltage between the cathode 14 and its associated reference electrode 50.

Figs. 9 and 10 correspond respectively to Figs. 3 and 8 and show potentiostatic (rather than galvanostatic) supply to the electrodes. The electrical supply and test apparatus 20' of Fig.9 is arranged for operation with a test device having no reference electrode, and so is suitable for use with a test device as shown in Figs. 1a and 1b. The apparatus 20' of Fig.10 is correspondingly

suited for operation with a test device as shown in Fig.6 and having two working electrodes and a reference electrode.

In Fig.9 the terminal 42 of the operational amplifier 40 is connected directly to the lead 26 and thence to the cathode 14. The terminal 44 is connected to lead 28 and thence to anode 16 via a resistor 60 across which the voltmeter 32 is connected. Terminal 46 is connected directly to lead 28.

The circuit of Fig.10 is identical to that of Fig.9 except the terminal 46 is connected to lead 54 rather than lead 28.

It will be understood that with a supply and test apparatus 20' as shown in Fig.9 or Fig.10 the voltmeter 32 will give a measure of the current passing through the electrodes. At a constant impressed voltage the steady state current attained will be smaller for a sample which is contaminated than for an uncontaminated sample. In a corresponding way to the galvanostatic systems previously described, the difference in current levels provides an indication of the presence, or otherwise, of bacterial growth.

CLAIMS:

1. A method of detecting microbiological contamination of a liquid or semi-liquid substance, the method comprising contacting spaced first and second electrodes with the substance in undiluted form, supplying an electrical current between the electrodes, monitoring the voltage or current between the electrodes, and producing a signal when the said voltage or current exceeds or falls below, respectively, a predetermined value indicative of contamination.

2. A method in accordance with Claim 1, which includes vibrating the test substance or the electrodes while testing is proceeding.

3. A method in accordance with Claim 2, wherein the frequency of vibration is about 5 vibrations per second.

4. A method in accordance with any preceding claim for detecting aerobic organisms, wherein the residual oxygen level within the liquid or semi-liquid substance is measured eletrochemically.

5. A method in accordance with Claim 4, wherein the liquid or semi-liquid is UHT milk.

6. A method in accordance with any preceding claim, which includes the step of incubating the liquid or semi-liquid substance prior to testing.

7. A method as claimed in any preceding claim, which includes, prior to supplying the said electrical current, supplying between the electrodes a conditioning pulse of an electrical current of substantially greater magnitude than the first said current.

8. A test device for carying out a method as claimed in any preceding claim, which comprises an insulating substrate, and first and second spaced carbon electrodes formed on the substrate by screen process printing using a carbon ink, at least end portions of the electrodes at one end of the test device being accessible electrically for connection to an electrical test apparatus by a severable electrical connection.

9. A test device in accordance with Claim 8, wherein the severable electrical connection has the form of a socket carrying first and second resilient electrical terminals adapted for making connection with the end portions of the electrodes when the said one end of the test device is inserted into the socket.

10. A test device in accordance with Claim 8 or Claim 9, wherein the substrate is in the form of a sheet of an insulating material.

11. A test device in accordance with Claim 10, wherein the insulating material is selected from the group comprising cartonboard, polyvinyl chloride, high impact polystyrene, polyethylene terephthalate, polycarbonate and polypropylene.

12. A method of detecting microbiological contamination of a liquid or semi-liquid substance, substantially as described with reference to the accompanying drawings.

13. A test device for use in a system for detecting microbiological contamination of a liquid or semi-liquid substance, the test device being substantially as described with reference to the accompanying drawings.